Nitrification in Acid Soils.

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Introductory.

Certain of the permanent grass plots at Rothamsted, which have been cut for hay and have received the same manurial treatment every year since 1856,* of late years have declined in yield, and the herbage has assumed an unhealthy condition. The plots affected are those to which nitrogen in the form of a mixture of ammonium chloride and sulphate is applied; the one receiving the highest amount of ammonium salts now carries a rank vegetation, consisting almost entirely of three species of grass —*Holcus lanatus*, *Alopecurus pratensis*, and *Arrenatherum avenaceum*— growing in coarse tufts with bare spaces between. The surface of these bare patches, which are spreading yearly, consists of a mat of peat-like decayed vegetation; a similar peaty formation is to be observed on the other plots receiving smaller amounts of ammonium salts.

In determining the nitrifying power of a number of Rothamsted soils,† Mr. S. F. Ashby observed that these grass soils failed to set up nitrification when small quantities were added to media suitable to the development of nitrates, indicating the comparative absence of the organisms causing nitrification. It was also noticed that the soil of these plots was distinctly acid, sufficiently so to redden blue litmus paper pressed against it in the moist state, a condition first observed by Voelcker‡ to be set up by the long-continued uses of ammonium salts on the arable soils of the farm of the Royal Agricultural Society at Woburn.

Inasmuch as the investigations of Warington and Winogradsky have shown that the process of nitrification can only go forward in the presence of a salifiable base and at once stops in an acid medium, it seemed possible that the observed decline in fertility of these plots might result from a cessation of nitrification due to the acid condition of the soil.

Though the soil of the grass plots is of the same origin and belongs to the same general type as that of the other Rothamsted fields—a stiff loam

* 'Phil. Trans.,' 1900, B, vol. 192, p. 139.
† 'Chem. Soc. Trans.,' 1905, vol. 85, p. 1158.
‡ 'Roy. Agric. Soc. Journ.,' 1904, vol. 64, p. 355.

approaching clay and including a good many unworn flint stones—it differs in one essential respect from them, in that it contains but a small proportion of calcium carbonate, a substance which is usually present in the surface layer of the Rothamsted soil to the extent of from 2 to 5 per cent. It has been shown* that the calcium carbonate at Rothamsted only exists in the surface soil and is of artificial origin, being due to repeated "chalking" during the eighteenth century and earlier with chalk rock drawn from 10 to 12 feet below the surface. As the land in question was in grass before the practice had become general, it cannot have been often subjected to the chalking process, at any rate the surface soil now contains little calcium carbonate, 0·5 per cent. at the most, and the lower depths show less than 0·1 per cent. On Plot 11–1, which has received the largest amount of ammonium salts, the proportion is less, though the chalk has not been entirely removed, 0·16 per cent. being still present in the surface layer. But that the acidity of the grass soils is a consequence of the comparative absence of calcium carbonate there can be little doubt, since the soil of the arable fields at Rothamsted, which contains from 2 to 5 per cent. of calcium carbonate, is still neutral, though the same amounts of ammonium salts have been applied over even longer periods.

To ascertain if the nitrifying organisms were present, small quantities of the soil were introduced into a suitable sterile culture medium containing an ammonium salt and other inorganic nutrients, together with a sufficiency of calcium or magnesium carbonate. Flasks containing 100 c.c. of such a solution were seeded with 0·2 to 0·5 gramme of soil, and were maintained at 30° C. with the usual precautions; after a month the liquids were tested for nitrates, nitrites, and ammonia. It is well known that nitrate or nitrite will be formed during such a period of incubation if any of the proper organisms have been introduced with the soil; as a rule, with normal soils the whole of the ammonia will be oxidised.

Samples were taken on six different occasions between October, 1904, and February, 1907, mostly from the surface soil only, but once down to 135 cm.; they were drawn from the continuously unmanured plot, from two plots receiving 400 lb. per acre per annum of ammonium salts, from one which received 600 lb. of ammonium salts, and from one plot to which sodium nitrate is applied in place of ammonium salts. Other samples were taken from the sections of these plots to which 2000 lb. per acre of quicklime had been applied in January, 1903.

On summarising the results, it appears that the soil of the unmanured plot from a depth of 10 to 15 cm. set up nitrification in seven out of eight

* Hall and Miller, 'Roy. Soc. Proc.,' B, 1905, vol. 77, p. 8.

trials, whilst with that from lower depths only failures were recorded, except in the case of two samples from 30 cm. The soil from Plot 4–2 (400 lb. ammonium salts) from a depth of 10 to 15 cm. only set up nitrification twice in nine trials, whilst that from lower depths proved active in six and inactive in four cases. The soil from Plot 9 [with the same ammonium salts], from a depth of 10 to 15 cm., failed to induce nitrification in 11 trials; with that from lower depths there were occasional successes. Soil from Plot 11–1, to which the largest quantity of ammonium salts is applied, never caused nitrification, except in the case of one sample from a depth of 45 cm., but the soil from Plot 14, which receives sodium nitrate instead of ammonium salts, induced a vigorous nitrification, except in the case of samples from depths of 105 and 135 cm., to which the nitrifying organisms are rarely likely to extend in heavy undisturbed grass land like that at Rothamsted.

The nitrifying organisms were more frequently found in the soil from the limed halves of the plots, though in the case of Plot 11–1 they were not detected in the upper layer.

These experiments indicated that the nitrifying organisms were present only sparingly in the soils which had received large amounts of ammonium salts, and that they were less abundant the more ammonium salts had been used, but that whenever the acidity of the soil had been reduced by the comparatively recent application of lime, nitrification was more frequently set up.

In order to verify these conclusions, two attempts were made to nitrify the soil itself in bulk. One or two kilogrammes of the moist soil, just as it came from the field, was spread out in a comparatively thin layer over water under a bell jar, so as to exclude dust and to maintain a moist atmosphere; after exposure in a dark part of the building during five weeks and two months respectively, the soils were rapidly dried and the amount of nitrates in them determined. The nitrates were also determined in another portion of the soil at starting, so as to ascertain the amount formed during the exposure under conditions favourable to nitrification. Of course, in sampling and manipulating such large quantities of material, it was impossible to prevent accidental introduction of the nitrifying organisms, but they would not affect the object of the experiment, which was to ascertain if the soils in their acid condition permit the nitrification process to go on. At the outset the soils were found to contain nitrates, but to a less extent than is usual. The quantities, however, varied from plot to plot with the amount of nitrogen supplied as manure; in one case, for example, the soil of Plot 11–1, receiving 600 lb. of ammonium

salts, contained 12·44 parts of nitric nitrogen per million of soil, whilst the soil of Plot 9, which received 400 lb. of ammonium salts, contained 6·17 parts nitric nitrogen per million, and that of the unmanured Plot 3 contained only 1·78 parts. Such a relationship between the amount of nitrates in the soil and the amount of ammonia supplied to each plot indicates that the nitrates have been formed in the soil instead of being derived from some extraneous source common to all the plots; this conclusion is confirmed by the further behaviour of the soils during exposure, since in nearly all cases the nitrates were found to have increased. The five weeks' exposure was attended by an increase of nitrates in all the surface soils except in that of Plot 11–1, to which the largest amount of ammonium salts is applied; similar, though smaller, increases were shown by all the other samples, except those taken from the depth of 113 to 135 cm., soil from which depth had not been observed to contain nitrifying organisms in the previous series of experiments. The two months' exposure (surface soils only) produced similar effects, the increase in nitrates being small in the soils from Plots 9 and 11–1.

Although these results show that the nitrifying organisms cannot be wholly absent from the soil of the plots rendered acid by the use of ammonium salts, yet the quantity of nitrates produced during the exposure, favourable as were the conditions for nitrification, would not be sufficient to supply crops grown on these plots with the amount of nitrogen they usually remove from the land. During the five weeks' period, the nitrates in the soil of Plot 9 increased by two parts per million; in that of Plot 11–1 by 0·64 part per million; assuming that these optimum rates were maintained during a whole year, the total amount of nitrates produced would only amount to 50 lb. and 16 lb. respectively of nitrogen per acre. Yet, in 1904, Plot 9 produced 70·4 cwt. of hay, containing 94·6 lb. of nitrogen, and Plot 11–1 98·2 cwt. of hay, containing 131 lb. of nitrogen, all of which must have been derived from the soil, as no leguminous plants are present in the herbage.

From these figures it is difficult to resist the conclusion that though nitrification may not be entirely suspended in these acid soils, it is so far reduced that the plants cannot obtain in the form of nitrates all the nitrogen they take up from the soil, but must draw the larger portion directly from the ammonium salts supplied as manure. Müntz* and Mazé† have shown that maize, another gramineous plant, can derive its supply of nitrogen directly from ammonium salts, but attempts made by the authors to grow

* 'Comptes Rendus,' 1889, vol. 109, p. 646.

† 'Ann. de l'Inst. Pasteur,' 1900, vol. 14, p. 26.

some of the grasses characteristic of the plots under discussion in a medium containing unnitrified ammonium salts have, so far, failed.

The next step in the enquiry was, if possible, to determine the nature and amount of the acid present in the soil, in order to ascertain if it presented any special features which would explain the continuance of nitrification in its presence.

No satisfactory method exists of determining the acidity of a soil, owing to the sparing solubility of the humic acids and the difficulty of removing acid from so extensive a surface as that presented by soil particles. Extracting the soil with lime water, or dilute solutions of alkaline salts, gives results that are too high, because of the errors introduced by the presence of phosphates, and the interactions which may be set up with the zeolitic double silicates, ferric hydrate, and other soil constituents. Since we were chiefly concerned with the acidity of the soil water, we have used water only, accepting the fact that the results will be too low. When large quantities of the undried soil from the most acid plot, 11–1, were rapidly washed with small quantities of hot water, an extract was obtained which showed an acidity equivalent to 1·71 grammes of hydrogen per million of dry soil. After this extraction the soil still remained acid to litmus paper, even though the washing was repeated with large quantities of water, so that the acidity observed can only represent the easily soluble acids, leaving the greater part of the "humic" acids still in the soil residue. The chlorides and sulphates present in the water extract were equivalent to 1·91 and 3·15 grammes respectively of hydrogen per million of soil, the two together being equivalent to about three times the acidity. The manures supplied every year to this plot would add sulphates equivalent to 5·2 grammes and chlorides equivalent to 2·3 grammes of hydrogen per million of soil; consequently there was rather less than one year's stock of chlorides and sulphates in the top layer of soil at the time of sampling.

Under ordinary field conditions, the amount of water present in the soil will vary between 10 and 25 per cent., so that the easily soluble acids extracted by washing would give rise to an acidity of the soil water varying between one-sixtieth and one-hundred-and-fortieth of normal.

It is hardly necessary to enquire into the nature of the free acid present; the acid water extract contains humates, chlorides, and sulphates, hence it follows that such external factors as relative mass, temperature, and concentration, will determine which of them will be in the condition of a free acid. When the extract is concentrated, a certain amount of "humic" acid is thrown out of solution; when it is brought to complete dryness, hydrogen chloride is given off as gas. The real question is, whether the acid

originates with the ammonium salts, or results from the attack of humic acid upon them. But free humic acid is not a normal product of decay in the soil; the unmanured plot, for example, is not acid; the "humus" of soil usually consists of calcium salts.* Further, the acidity of the grass plots under discussion increases with the amount of ammonium salts annually applied, so that it is to the ammonium salts we may look for the origin of the acid. Moreover, as the amount of freely soluble acid in the soil is of the same order of magnitude as the sulphuric and hydrochloric acids contained in one year's application of ammonium salts, we can suppose that it has been recently split off from them, but it does not accumulate from year to year, because it slowly acts upon the calcium humate present, forming the sparingly soluble free humic acid, and calcium sulphate and chloride which are removed in the drainage water. In confirmation of this opinion, it was found that a solution of ammonia would dissolve considerable quantities of humic acid from the soil of Plot 11–1, though in the case of normal soils it is necessary to break up the humates with hydrochloric acid before they will yield any humic acid to ammonia solution.

The next step in the enquiry was to find if any agency existed in the soil capable of splitting up ammonium salts so as to set free the acids therein. In a previous paper,† two of the authors have shown that no free acid results from purely chemical or physical interactions of ammonium salts and the soil constituents. Neither the double silicates nor the calcium humate in the soil exercise a selective absorption of the base to set free the acid, nor is there hydrolysis of the salt followed by adsorption or evaporation of the ammonia; the action between ammonium salts and the soil constituents is always in the nature of a double decomposition attended by no change in the neutrality of the medium. Being thus constrained to look for a biological explanation of the acidity in the grass soils, nutrient solutions were made up, containing, besides dextrose and the usual nutrient salts, ammonium sulphate or chloride as the only nitrogenous compound. These were rendered faintly acid and seeded either with the fresh soil or with a cold water extract from one of the acid grass plots. A vigorous crop of moulds and other fungi soon appeared, at the same time the liquid became markedly acid. The dominant organisms isolated in this way were forms of *Penicillium glaucum* and a mould allied to *Mucor*, and when pure cultivations of any of these species were seeded into solutions containing a carbohydrate and ammonium salts, the organisms were found to grow and to remove ammonia from the solution, which, at the same time,

* Hall and Gimingham, 'Chem. Soc. Trans.,' 1907, vol. 91, p. 677.

† Hall and Gimingham, *loc. cit.*

became acid, the acidity being sometimes greater and sometimes less than the equivalent of the ammonia withdrawn. The degree of acidity attained in this way lay between one-fiftieth and one-two-hundredth of normal; usually, growth stopped at about one-eightieth normal. It is significant that this is about the same degree of acidity as was found to prevail in the soil water of the acid grass plots. That the acidity is due to the attack of the organisms on the ammonium salts is seen from the fact that the culture solutions remain neutral when asparagin is substituted for the ammonium salts, or become alkaline when peptone is used as the source of nitrogen. Considering the abundance of these moulds in the peaty surface soil of the acid grass plots, and the rapidity with which they will work (*in vitro* the maximum acidity is attained in a week or ten days, just as the grass plots are most acid a few days after the application of the ammonium salts), the authors conclude that they are the active agents in producing the acidity of the soil of the grass plots in question. That the change in the ammonium salts takes this direction is due to the initial acid state of the soil; in such an acid medium the moulds have free play, while the nitrifying bacteria attacking ammonium salts in normal soils are checked or even suppressed. Doubtless the present acidity grew up gradually and locally as the original small stock of calcium carbonate in the soil became removed by the solvent action of the ammonium salts.

Having thus reached the conclusion, both by direct examination and consideration of origin, that the acidity observed in the soil is due simply to sulphuric and hydrochloric acids derived from the ammonium salts, how it is possible for nitrification still to go forward, since all investigators are agreed that the action is stopped as soon as the medium ceases to be neutral or faintly alkaline? To test the point further, water extracts were made from the soil of Plot 11–1, and concentrated until they represented the normal soil water, a little ammonium sulphate was added, and they were seeded with a strongly nitrifying extract from a garden soil and put to incubate. Check solutions were treated in the same way, but were first neutralised by the supply of calcium and magnesium carbonates. The nitrates were determined in portions of the soil extract at the outset and after three weeks' incubation, when it was found that no nitrification had taken place except in the neutralised solutions. Dilute cold water extracts from the acid soil also refused to nitrify except when neutralised.

When the extracts were not neutralised they were always invaded by moulds, which were especially abundant in the cold water extracts.

Thus there is found in the soil a degree of acidity which will inhibit nitrification *in vitro*; even a water extract of the soil will not nitrify, ye

nitrification, though considerably reduced, does go on in the same soil under field conditions. The explanation is probably to be found in the want of uniformity in a solid material like soil; though the soil of Plot 11–1 is on the whole so acid, the analyses show that it still contains 0·16 per cent. of finely divided calcium carbonate, each of the grains of which would be a centre for nitrification, though the process might not be going on outside the region kept neutral by the calcium carbonate. Further, all chemical actions in a soil must be much modified and localised by having to take place in the thin film of water clinging by surface tension to the soil particles; diffusion would be very slow, so that interactions of a quite opposite nature might be taking place at the same time and within a very short distance.

This nitrification in acid soils is not unlike the case investigated by Chick,* who found that sewage nitrifies freely in passing through a coke bed, though the amount of organic matter dissolved in the sewage either entering or leaving the filter bed is sufficient to inhibit the nitrification of the liquid *in vitro*.

The authors conclude, then, that nitrification will continue in the Rothamsted soils as long as any particles of available calcium carbonate remain disseminated through them; the continuance of the present manurial treatment, however, must eventually reduce the soil to a uniformly acid condition, when nitrification may be expected to cease, just as happens in the case of peat or other naturally acid soils. The unhealthy condition of the plots may be set down to the fact that their acidity checks the work of the nitrifying and other bacteria and promotes instead the development of moulds. The moulds must compete with the grass for the nitrogen compounds supplied as manure, but whether they injure the crop in other ways is under further investigation.

Experimental.

A. *Soil and Manure.*

The following table shows the manurial treatment of the plots in question every year since 1856, together with the average yield of hay per acre and the nitrogen removed therein.

Plot.	Ammonium sulphate.	Ammonium chloride.	Super-phosphate.	Potassium sulphate.	Sodium sulphate.	Magnesium sulphate.	Average yield of hay.	Nitrogen per acre.
	lb.	lb.	cwt.	lb.	lb.	lb.	cwt.	lb.
3	—	—	—	—	—	—	21·6	33·9
4–2	200	200	3·5	—	—	—	35·7	66·1
9	200	200	3·5	500	100	100	54·4	76·8
11–1	300	300	3·5	500	100	100	66·8	107·2

* 'Roy. Soc. Proc.,' 1906, B, vol. 77, p. 241.

To one-half of each of the plots, 2000 lb. per acre of ground quicklime was applied in January, 1903, the application being renewed in 1907.

Samples of soil taken in October, 1906, from the unmanured Plot 3, and from Plot 11–1, which receives the largest amount of ammonium salts, showed the following proportions of calcium carbonate, as calculated from the carbon dioxide evolved on treatment with hydrochloric acid.

Depth. inches.	Plot 3.	Plot 11–1.
0— 9	0·516	0·16*
10—18	0·206	0·151
19—27	0·092	0·106
28—36	0·087	0·141

B. *Examination of the Soil for the Nitrifying Organism.*

From 0·2 to 1 gramme of the soil under examination was added to 100 c.c. of a culture solution made up as follows:—

Ammonium sulphate..................	0·5	gramme per litre
Monopotassium phosphate	0·25	,,
Magnesium sulphate (cryst.).........	0·15	,,
Sodium chloride........................	0·5	,,
Ferrous sulphate	0·1	,,

together with about 0·2 gramme of a mixture of sterilised magnesium and calcium carbonate. The solutions were in Erlenmeyer flasks, and had previously been sterilised in the usual way; they were then incubated for at least a month at a temperature of 30°, the contents being gently shaken two or three times during that period. At the end an examination of the clear liquid was made for nitrates with diphenylamine, for nitrites with metaphenylene-diamine, for ammonia with Nessler's reagent.

The soil samples were prepared as follows:—

In four series holes were dug in the field and sterile brass tubes 1 inch in diameter were forced into the sides, after breaking away some soil to expose a clean surface. The tubes were carried to the laboratory, the contents forced out into dishes and partly dried over strong sulphuric acid, suitable precautions being taken to avoid external contamination. The partly dried soil was then roughly powdered and stones removed by passing through a 1 mm. sieve, sieve and mortar being sterilised each time. By a suitable spoon a quantity found by trial to be approximately 0·2 gramme

* Since the above was written the authors have seen reason to suppose that some of the carbon dioxide, evolved on treating soils of this character with hydrochloric acid, comes from the organic matter. Several other processes agree to make the calcium carbonate in the surface layer amount only to 0·04 per cent.—*Note added March* 19, 1908.

was introduced into the culture solution. For the last two series the sample of soil was extracted with an auger 2 inches in diameter having a slot in the side; at the selected depth in the slot a clean surface of soil was exposed and a small sample, approximately 0·5 gramme, was picked out with a sterile spatula and introduced into the culture flask, the operation being carried out in the field.

Number of Times reacting after Four Weeks' Incubation.

Depth. cm.	Unlimed portion.		Limed portion.	
	Nitrate.	No nitrate nor nitrite.	Nitrate.	No nitrate nor nitrite.
		Plot 3.—Unmanured.		
10—15	7	1	1	0
30	2	3	1	0
45	0	1		
75	0	1		
105	0	1		
135	0	1		
		Plot 4–2.—Ammonium Salts and Superphosphate only.		
10—15	2	7	1	2
20—30	5	1	1	1
45	1	0	0	1
75	0	1	0	1
105	0	1	0	1
135	0	1	0	1
		Plot 9.—400 lb. Ammonium Salts and Complete Minerals.		
10—15	0	11	3	4
20—30	2	3	2	1
50	1	0	1	0
70—75	2	0	0	1
105	0	1	0	1
135	0	1	0	1
		Plot 11–1.—600 lb. Ammonium Salts and Complete Minerals.		
15	0	2	0	2
30	0	2	0	2
45	1	0	0	1
75	0	1	1	0
105	0	1	0	1
135	0	1	0	1
		Plot 14.—550 lb. Sodium Nitrate and Complete Minerals.		
15	1	0		
30	1	0		
45	1	0		
75	1	0		
105	0	1		
135	0	1		

C. *The Nitrification of the Soils in Bulk.*

Large samples of the soil down to six depths of 9 inches were taken in October, 1906, and one portion of each was at once dried at a temperature of

70° to 80°, and the other placed in a thin layer under a bell jar over water. At the end of five weeks these second portions were similarly dried, and the two sets of samples, after removal of stones and reduction to a powder, were washed on a pressure filter with successive portions of water; the nitrates were determined in the extract by reduction and distillation of the ammonia. In the second trial the surface soil only, to a depth of 9 inches, was sampled in July, 1907; the exposure, made in the same way, was on this occasion continued for two months.

The following table shows the amount of nitric nitrogen per million of dry soil both before and after exposure, also the differences between the two sets of figures to show the amount of nitrates formed during the exposure.

Rothamsted Grass Soils. October, 1906.

Nitrogen as Nitric Acid per Million of Dry Soil.

Plot.		1st 9 inches.	2nd 9 inches.	3rd 9 inches.	4th 9 inches.	5th 9 inches.	6th 9 inches.
				In Soil before Exposure.			
3	Unlimed	1 ·06	—	0 ·54	—	2 ·19	1 ·33
4–2	Limed...	4 ·33	—	0 ·88	2 ·92	—	0 ·39
4–2	Unlimed	3 ·75	1 ·39	1 ·07	1 ·13	1 ·09	0 ·67
9	Limed...	2 ·25	1 ·75	1 ·35	0 ·79	1 ·08	2 ·00
9	Unlimed	2 ·38	1 ·11	0 ·90	1 ·20	0 ·47	0 ·58
11–1	Limed...	2 ·38	0 ·92	1 ·09	0 ·79	0 ·92	0 ·92
11–1	Unlimed	3 ·00	1 ·20	1 ·39	1 ·08	1 ·83	1 ·83
14	Unlimed	4 ·75	1 ·33	2 ·60	0 ·44	0 ·83	0 ·53
				In Soil after Exposure.			
3	Unlimed	1 ·41	0 ·97	0 ·67	1 ·08	0 ·96	0 ·63
4–2	Limed...	6 ·12	2 ·04	1 ·00	2 ·00	3 ·66	0 ·36
4–2	Unlimed	5 ·96	2 ·30	1 ·17	1 ·15	1 ·04	0 ·67
9	Limed...	2 ·70	2 ·54	1 ·30	1 ·32	1 ·25	1 ·49
9	Unlimed	3 ·42	1 ·15	0 ·77	1 ·39	1 ·14	0 ·79
11–1	Limed...	2 ·67	2 ·10	1 ·53	1 ·12	0 ·83	0 ·58
11–1	Unlimed	2 ·96	2 ·08	1 ·63	1 ·46	1 ·92	0 ·92
14	Unlimed	8 ·42	1 ·50	2 ·50	1 ·09	0 ·79	0 ·53
				Gain or Loss by Exposure.			
3	Unlimed	+0 ·35	—	+0 ·13	—	−1 ·23	−0 ·70
4–2	Limed...	+1 ·79	—	+0 ·12	−0 ·92	—	−0 ·03
4–2	Unlimed	+2 ·21	+0 ·91	+0 ·10	+0 ·02	−0 ·05	0
9	Limed...	+0 ·45	+0 ·79	−0 ·05	+0 ·53	+0 ·17	−0 ·51
9	Unlimed	+1 ·04	+0 ·04	−0 ·13	+0 ·19	+0 ·67	+0 ·21
11–1	Limed...	+0 ·29	+1 ·18	+0 ·44	+0 ·33	−0 ·09	−0 ·34
11–1	Unlimed	−0 ·04	+0 ·88	+0 ·24	+0 ·38	+0 ·09	−0 ·91
14	Unlimed	+3 ·67	+0 ·17	−0 ·10	+0 ·65	−0 ·04	0

July, 1907. First 9 inches of depth only.

Plot.	At starting.	After exposure.	Gain by exposure.
3	1 ·78	2 ·51	0 ·73
4–2	3 ·39	9 ·01	5 ·62
9	6 ·17	6 ·79	0 ·62
11–1	12 ·44	13 ·38	0 ·94
14	10 ·77	13 ·03	2 ·26

Two kilogrammes of an air-dried sample of the soil from Plot 11–1 were extracted with successive quantities of hot water and the extract concentrated to a volume of 500 c.c.; 50 c.c. portions of this were placed in the usual flasks for nitrification, 5 c.c. of a 1-per-cent. solution of ammonium sulphate was added, and each was seeded with 5 c.c. of a cold water extract from a garden soil in a good state of fertility. The object was to ascertain if the acidity of such a medium, which would represent the solution in the soil when it contains 25 per cent. of water, would be sufficient to inhibit nitrification after a fresh stock of the organisms had been supplied. The extraction of the dried soil had, however, not been very effective, and the initial acidity was only equivalent to 0·6 c.c. of $n/10$ alkali, or a little more than one-thousandth normal. In the check flasks the acidity was removed by the addition of a mixture of calcium or magnesium carbonates, and they were all incubated for three weeks at 30° C. Determinations were made of the nitrates before and after incubation, with the following results:—

50 c.c. Soil Extract + 5 c.c. Soil Extract + 5 c.c. 1-per-cent. $(NH_4)_2SO_4$.

		Nitrogen as nitrates. milligrammes.
1, 2, 3	No calcium or magnesium carbonate, incubated	0·95
4, 5, 6	With calcium and magnesium carbonates, incubated	2·38
7, 8	Not incubated	1·25

There was no formation of nitrates except when the acid had been neutralised by the calcium and magnesium carbonates. In 1, 2, and 3 weak growths of mycelium were visible, but none in the neutral extracts, 4, 5, 6.

In a further experiment fresh soil was brought from Plot 11–1 and extracted rapidly with cold water; the extract had a slight acidity, equal to 0·6 c.c. $n/10$ acid per 100 c.c. Portions of 50 c.c. were taken and 5 c.c. of 1-per-cent. ammonium sulphate were added to each; in some cases the extract alone was incubated for three weeks, with or without earthy carbonates; in others, 5 c.c. of a garden soil extract prepared as before was added to introduce the nitrification organisms. Nitrates were determined after a month's incubation, and in the checks before starting, with the following results:—

50 c.c. Soil Extract + 5 c.c. 1-per-cent. $(NH_4)_2SO_4$.

		Nitrogen as nitrates. milligrammes.
1 and 2	Not incubated	0·25
3 „ 4	No calcium and magnesium carbonate, incubated	0·23
5 „ 6	+ Calcium and magnesium carbonate, incubated	0·31
7 „ 8	+ 5 c.c. garden soil extract, no calcium and magnesium carbonates, incubated	0·39
9 „ 10	+ 5 c.c. garden soil extract, + calcium and magnesium carbonates, incubated	6·83

Again there was no formation of nitrates except when the acidity of the medium had been neutralised by the calcium and magnesium carbonates.

Flask 4 showed a heavy crop of moulds in fructification, 7 and 8 showed mycelium in the liquid, 10 one or two colonies of a bacterium on the surface.

D. *The Nature and Extent of the Acidity.*

Fresh soil to the depth of 9 inches was taken in March, 1907, a month after the application of the ammonium salts, and about 7 kilogrammes of the soil as it came from the field was extracted on a pressure filter with successive portions of hot water. No attempt was made to remove all the acid, as previous trials had shown this to be impossible with water alone; the soil after extraction still reddened blue litmus paper. The extract showed an acidity equivalent to 1·71 grammes hydrogen per million of dry soil. Sulphates and chlorides were also determined in the extract as follows:—

Chlorides	equivalent to	1·91	grammes hydrogen	per million soil.
Sulphates	"	3·15	"	"

When the soil was extracted with a fifth normal solution of sodium chloride, the acidity of the extract amounted to 30 grammes hydrogen per million of dry soil; substituting potassium nitrate for the sodium chloride, the acidity was equivalent to 42·5 grammes hydrogen per million of dry soil. In these two latter cases humic acid, insoluble in the water extract, was also being estimated.

Another sample was taken in November, 1907, and extracted in the same manner with the following results:—

Acidity	equivalent to	1·02	grammes hydrogen	per million soil.
Chlorides	"	0·35	"	"
Sulphates	"	1·60	"	"

At this latter date a sample was taken from Plot 9 and gave the following results:—

Acidity	equivalent to	0·36	grammes hydrogen	per million soil.
Chlorides	"	0·47	"	"
Sulphates	"	1·69	"	"

In this case some of the humic acids dissolved in the original extract were thrown out during the evaporation of the extract, hence the acidity measured is a little low. The acidity of the soil persists after drying in the air, but is measurably reduced when the soil is dried in the steam oven.

If these measured acidities are calculated on the proportion of water usually found in the soil, which will vary between 10 and 25 per cent. of its dry weight, the following results are obtained:—

Approximate Acidity of Soil-water. Fractions of normal.

	When soil contains 10 per cent. water.	When soil contains 25 per cent. water.
Plot 11–1, March, 1907	1/60	1/150
Plot 11–1, November, 1907...	1/100	1/250
Plot 9, November, 1907	1/300	1/700

To ascertain if the acid soils contain any free humic acid, 10 grammes of the soil of Plot 11–1 were extracted with 500 c.c. of 4-per-cent. ammonia solution, and the amount of organic matter going into solution was determined. On a second 10 grammes the usual process of determining "humus" was followed; the soil was treated with hydrochloric acid, and the acid washed away before extracting with the ammonia solution. Parallel experiments were made with a prairie soil rich in humus but neutral, since it contained 3·5 per cent. of calcium carbonate. The following results were obtained:—

	Organic matter soluble in ammonia.	
	Without acid treatment.	After preliminary acid treatment.
Plot 11–1	3·54 per cent.	4·51 per cent.
Prairie 0–4	Nil	4·65 „

The prairie soil yielded no humic acid to ammonia, unless the humates it contained had first been decomposed by hydrochloric acid, whereas the soil of Plot 11–1 contained 3·54 per cent. of humic acid immediately soluble in ammonia.

E. *Liberation of Acid from Ammonium Salts.*

To ascertain if any of the organisms in the soil of these acid plots were capable of setting free ammonia from its salts, small Erlenmeyer flasks, containing a solution made up of 2 grammes ammonium chloride, 0·5 gramme monopotassium phosphate, 0·25 gramme magnesium sulphate, 1 gramme sodium chloride, and 10 grammes of glucose per litre, were sterilised, inoculated with the soil or soil extract, and incubated at 21° C. After seven days a vigorous growth of various moulds was observed, and the medium was found to be distinctly acid. In mixed cultures of this kind the acidity rose from about $n/166$ to $n/80$ in a week, and then remained constant. Sub-cultures were made and the organisms isolated in the usual way: the dominant species were forms of *Penicillium glaucum* and a *Mucor* (?), which differs in several respects from those already known, and does not appear to have been described.* Samples taken from Plots 11–1, 9, and 4–2 at different times during the spring and summer showed the same dominance of *Penicillium* and the *Mucor*. Various other species of

* This mould has since been identified by Professor A. F. Blakeslee as *Zygorhynchus Moelleri*, Vuill.—*Note added March* 19, 1908.

Mucor, *Trichoderma*, and *Acrostalagmus* have also been observed, but the description of the fungus flora present in the soil of these plots will be given elsewhere. The essential point is that both the dominant species, *Penicillium* and the *Mucor*, and several of the others examined, when grown into a nutrient medium containing a carbohydrate and an ammonium salt, draw the nitrogen they require from the ammonia and leave the acid in a free state. This production of acid by *Penicillium* growing in a medium containing an ammonium salt has already been observed.* The following experiments will serve to illustrate the extent to which the action takes place:—

Experiments with Mucor sp. ?—Flasks were made up with 60 c.c. of a nutrient solution containing varying amounts of ammonium chloride and cane-sugar as under; the flasks were incubated at 21° C., and the acidity determined after 34 days, with the results set out in the last columns:—

	NH_4Cl.	Cane-sugar.	Gain of acidity as c.c. $n/10$ acid.	Percentage of ammonium chloride converted.
	gramme.	grammes.		
A 1	0·6	1·2	11·6	13·9
A 2	0·6	0·6	8·0	7·2
B 1	0·3	1·2	8·1	14·5
B 2	0·3	0·6	6·9	12·3
C 1	0·06	1·2	9·1	81·7
C 2	0·06	0·6	8·5	75·0

The highest acidity observed at the end was equivalent to about a $n/50$ solution of free acid.

When a small quantity of asparagine was also added to the nutrient solutions the increase of acidity was negligible; the organism satisfied its requirements for nitrogen from the asparagine in preference to the ammonium chloride, which, in consequence, remained undecomposed.

Experiments with Penicillium.—One of the forms of *Penicillium* was seeded into a solution containing 1 per cent. glucose, 0·25 per cent. ammonium chloride, and the usual nutrient salts. Growth was fairly vigorous and was stopped after 14 days, when the yellow solution was filtered off and tested for acidity and ammonia, showing the following results for 100 c.c. of the original solution:—

	Acidity as c.c. $n/10$.	Nitrogen as ammonia.	Ammonia removed.
Check	0	0·063	
A	5·5	0·059	0·004 = 2·8 c.c. $n/10$ acid
B	5·2	0·058	0·005 = 3·5 ,,

The acidity in this case has been increased, though not to the extent

* Kohn and Czapek, 'Beitr. Chem. Physiol. Path.,' 1906, vol. 8, p. 302.

found with the *Mucor*; at the same time, ammoniacal nitrogen has been withdrawn from solution in amounts accounting for rather more than half of the observed acidity.

Another set of observations made by Dr. H. B. Hutchinson gave the following results:—

Acidity produced and ammonia withdrawn from solution containing 1 per cent. dextrose and 0·2 per cent. ammonium sulphate, during 21 days' growth of various moulds isolated from Plot 11–1.

Cubic centimetres of Tenth Normal Acid per 100 c.c. Culture Medium.

Organism.	Acidity.	Acid equivalent of ammonia withdrawn.
Mucor sp.?	6·0	11·1
Mucor I	4·1	5·6
Mucor II	5·44	9·6
Penicillium	7·5	12·4
Acrostalagmus	10·1	11·9
Trichoderma I	11·3	13·9
Trichoderma II	12·54	13·6

In these cases there was always a greater amount of ammonia withdrawn from solution than was equivalent to the acidity measured, which, as before, amounts to a concentration of from $n/200$ to $n/70$ in the final solution. It is further significant that the degree of acidity developed in the culture solutions is of about the same order, between fiftieth and two-hundredth normal, as that which was found to exist in the soil water of the field plots.

Summary.

In the soil of certain of the permanent grass plots at Rothamsted, which is distinctly acid in consequence of the long-continued use of ammonium chloride and sulphate as manure, nitrification is greatly reduced, and the nitrifying bacteria are only found sparingly. In bulk, nitrification still goes on slowly, despite the acidity of the soil. Water extracts of the soil will not permit of nitrification unless they are previously neutralised. The amount of nitrate produced would not be sufficient for the nitrogen taken up by the crop, which must, in the main, utilise the ammonium salts without previous change. The acidity is chiefly due to sparingly soluble "humic" acids; free hydrochloric and sulphuric acids are also present, because the soil extract contains soluble acid in quantities comparable to the amount of chlorides and sulphates also present, and to the ammonium sulphate and chloride annually supplied as manure.

The acidity is not brought about by purely chemical or physical actions of the soil upon the ammonium salts, but by various micro-fungi which are able to remove ammonia from a solution of its salts and set free the acids

with which it was combined, the acidity attained in this way being equivalent to that of the soil water on the acid plots.

The authors attribute the continuance of the nitrification in these soils to the irregular distribution of the materials composing them; though acid as a whole, they still contain some calcium carbonate, each of the particles of which forms a centre for the nitrification process. The decline in fertility of the acid plots may be attributed to the repression of the normal bacterial activities of the soil and the encouragement of the growth of moulds.

The Origin and Destiny of Cholesterol in the Animal Organism. Part I.—*On the so-called Hippocoprosterol.*

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(From the Physiological Laboratory of the University of London.)

Introductory.

Since the discovery of cholesterol by Conradi in 1775, and its analysis by Chevreul in 1815, it has been found to be very widely distributed in the animal and in isomeric forms in the vegetable kingdom. It is found in small quantities in all protoplasmic structures, in blood, bile, sebum, and similar oily excretions of the skin, and is an especially abundant constituent of the white substance of brain and of the medullary sheath of nerve. But, although a considerable amount of work has been done, we have little or no definite knowledge of its physiological functions, and it is only in very recent times that a small glimmering of light has been thrown on its chemical constitution.

In 1862, Austin Flint* published a series of experiments by which he attempted to show that cholesterol is always more abundant in the blood coming from the brain than in the blood of the general arterial system, or in the venous blood from other parts; that its quantity is hardly appreciable in venous blood from the paralysed side in hemiplegia, and that it is

* "Experimental Researches into a new Excretory Function of the Liver," 'American Journal of Medical Sciences,' Philadelphia, 1862, new series, vol. 44, and "Recherches Expérimentales sur une Nouvelle Fonction du Foie," Paris, 1868.